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Genesis: A Conscious Approach to Immortality

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ABSTRACT

Our ability for genius progression is evident in everything that surrounds us; the cost of that success needs careful consideration. The ability to reconstruct and manipulate genomes to analyse differential effects of specific genetic pathways is now at a level that we can recreate a genome encompassing everything essential for complete human production with the potential of improving it towards a more stable organism. Precise genome editing allowing insertion of peptide drug sequences to be transcribed simultaneously with their corresponding target molecules, cells, tissues and organs is now possible, thus enabling targeted delivery and functioning with decreased deleterious effects controlled genetically through usual feedback mechanisms eradicating the need for timely (parenteral or otherwise) medication. The ability to test and introduce these genetic improvements with*in silico* and *in vitro* model systems (human and animal stem cell cultures, tissue engineering and synthetic biology) void of (un)-ethical euthanasia based animal studies is the next step towards our guardianship of not just our own but of all life.

1. INTRODUCTION: GENETIC MODIFICATION TO ALTER PHARMACEUTICAL DEPENDENCE

"Man is not just a biological but a psychosocial organism"-Sir Julian Huxley[1]. Progress and biological improvement have been selected through natural, psychological and social pressures, leading to the persistence, improvement, and multiplication of some

"dominant" strains, species and patterns of organization, coupled with the reduction and extinction of other "recessive" types [1, 2]. The genetic material in any organism defines its range of potentialities [3]. Changes or mutations in this genetic material change the potentialities of the organism depending on their survival value towards improving existing or developing new advantageous phenotypes[3-5].

Can we introduce beneficial sequences, such as drug/peptide sequences, to be transcribed simultaneously with target proteins allowing precise manufacture/delivery when and where necessary, controlled by the same feedback loops and genetic 'switches' for the target protein, eradicating uncontrolled delivery of drug side-effects?

Several disease types exist today corresponding to genetic sequence and consequent protein function.For example, despite its conserved function of transporting thyroid and retinol around the body for growth and development, transthyretin (TTR) can misfold and form insoluble aggregates, fibrils and plaques that are deposited around the body affecting normal functioning, causing disease (amyloidosis)[6-8]. The distribution of these proteins varies and biological membrane barriers such as the blood-brain barrier can make delivery of stabilising drugs an issue. There are several peptides and non-steroid anti-inflammatory drugs (NSAIDs) that have been tried and tested to help stabilise essential proteins from misfolding, but efficient targeted delivery of these therapeutic peptides is limited and in some cases have adverse side effects due to uncontrolled distribution [9-11].Parenteral (dosage forms, injection or infusion) are the most commonly employed method of administration for therapeutic proteins and peptides, however requirement of frequent injections result in poor patient compliance [12, 13]. Non-invasive drug delivery routes such as nasal, transdermal, pulmonary, oral, and ocular routes offer some advantages but delivery is challenging and limited due to the presence of proteolytic enzymes, immunogenicity, destabilising pH changes, large molecular size, and poor permeation across biological membranes [12, 14-19]. Nanostructured peptide carrierscan improve permeability and utilise pH-sensitivity and solubility differences improving delivery, but complications remain [12, 20].

Introduction of base analogues, purine or pyrimidine, into replicating DNA or RNA, resulting in stabilising or deleterious effects of the genetic sequence can result in the increase or loss of specific protein production, the effective functioning of a protein, or altered physical and biochemical properties such as thermal stability or changed sensitivity to molecules [3, 21-31]. Studies with alternative nucleic acids (ANA, GNA, HNA, PNA and TNA) have been shown to direct template synthesis with equal or better efficiency than DNA or RNA making them improved alternates for genetic transmission/replication/evolutionary stability [32-36]. Small-interfering RNAs (siRNAs), micro-interfering RNAs (miRNA), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered, regularly interspaced, short palindromic repeat (CRISPR/CRISPR-associated Cas-9 systems) comprise a powerful class of specific targeted gene therapy genome editing techniques [37-45].

Using current gene editingknowledge, could we introduce the stabilising drug peptide sequences in regions closely associated with the corresponding protein sequence so that transcription and translation of both the drug and the protein occur simultaneously, ensuring maximum delivery/effect of the drug-protein complex. No need for timely doses as the drug would be produced at the same time as the proteins functions are required through the body's usual feedback mechanisms.The stabilising peptide would complex with the target protein as synthesis occurs, decreasing misfolding probabilities, and allowing full efficient functioning of the protein. The problem of getting drug peptides across biological membrane barriers (mucociliary, pulmonary, intestinal, blood-brain, dermal) would also be overcome. These kinds of genetic advances would decrease dependence on expensive pharmaceutical and medical procedures if allowed to be tested. There are several social and ethical limitations revolving around research regulated by the Food and Drug Administration (FDA), the Centres for Disease Control and Prevention, Department of Health and Human Services (DHHS), National Center for Complementary and Alternative Medicine (NCCAM), National Institutes of Health (NIH), National Library of Medicine, National Science Foundation, Office of Disease Prevention, Therapeutic Goods Administration (TGA), International Conference on Harmonisation (ICH), United Nations Health Care Organization (UNHCO), World Health Organization (WHO), World Trade Organization (WTO), among other international organisations that uphold strict and essential regulatory laws. The potential to test these genetic modifications through *in silico*simulations*, in vitro* embryonic and adult cell culture technologies, tissue engineering and regenerative medicine, coupled with a humane approach, is possible.

2. A CONSCIOUS APPROACH TO GENETIC ENGINEERING

A vast amount of simulations analysing differential gene modifications to determinebeneficial or deleterious implications can be gathered as a first stage proof-of-concept application prior to any testing using *in silico* genetic computer modelling [43, 46-53]. *S*imulation studies have been extensively used for scenarios of RNA-DNA-protein evolution [50-52]. Simulations with promise could then be introduced to *in vitro* systems, such as cell culture and stem cell technologies, embryonic developmental studies (fish, amphibian, etc), all void of any invasive unethical animal model experiments that result in the programmed death of the organism [40, 41, 43, 46, 49, 52, 54, 55].

Bacterial and virus systems are well known to be advantageous over more complex systems in detailing correlation of nucleic acid base sequence with protein amino acid sequence, structure and function [25, 56-58]. Gene transfer vectors offer high levels of transduction efficiency with unique advantages for gene therapy applications. Replication-competent retrovirus can permanently integrate into the genome of infected cells and requires cell division for transduction [59]. Lentiviruses transduce not only proliferating cells but also non-proliferating cells and lead to prolonged gene expression. Adenoviral vectors can deliver genes to

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dividing and non-dividing cells with very high transduction efficiency [60]. Adeno-associated viruses (AAV) can infect dividing and non-dividing cell types and cause no pathologic/toxicity effects but can transfect only a limited size of DNA (<5kb) [61-63]. Herpes simplex virus vectors have the advantage of being able to infect non-dividing cells, and have the capacity to carry large exogenous DNA (~40kb), but cytotoxicity and maintenance of transgene expression can limit their therapeutic applications [37, 59]. Plasmid vectors do not integrate in to the host genome and are lost during cell division. Viral vectors pose issues of endogenous detrimental recombination, oncogenic and immune effects [37]. These technologies could be used to manipulate genes within cell cultures from various tissues and organs to analyse differential effects void of *in vivo* animal testing [64].

Stem cell cultures can be utilised to analyse effects of genetic modifications from embryonic development through to adult growth[65]. These modifications could be analysed in stem cells and adult cell cultures in all tissue types from virtually any animal existent. Comparative biology helps elucidate essential modifications creating different phenotypes not easily seen otherwise.Vast comparisons of developmental and disease pathways through initial stem cell differentiation and proliferation to similar or differential effects in adult cell cultures can be done*in vitro* and void of actual oppression of the animal to disease conditions. Cell culture studies can be carried out over short periods of time from a few hours to days, or even years[58]. Routine genetic sequencing steps between studies can be used to ensure correct mutation of specific chromosomal/genetic regions is maintained or altered during studies.

External fertilization of fish provides a clear window for developmental process and increased accessibility for manipulation [66]. Extensive research of *in vitro* fertilisation on other mammals including rats, mice and humans is elucidating mechanisms essential to growth and development [67-69] These *in vitro* potentials allow external analysis of embryonic developmental through to adult maturation in wild-type and mutant genomes void of subjecting an adult organism to the possible deleterious effects [70, 71]. Storage of gametes, embryos, and stem cells across all species possible in medical, veterinary, and agricultural research, provides virtually limitless potential for these technologies to be used for comparative genetics [72]. These characteristic differences in animal growth and reproduction cycles analysed through stem cell and tissue engineering technologies provide larger necessary mapsto analyse differential genome variations.

Synthetic cell biology provides an alternate versatile framework for studying genotype-phenotype platforms, with vast growth in synthetic biology from self-replicating DNA to enzymatic replication to protocell compartmentalisation [73, 74] Studies in nucleic acid chemistry directed evolution and membrane biophysics have brought the prospect of a simple synthetic cell with life-like properties such as growth, division, heredity and evolution within reach [73, 75-79]. Genetic modifications within simple synthetic cell prototypes, provide a less convoluted framework of growth and developmental processes than tissue and cell culture studies, allowing strong cooperative analysis of disease and developmental pathways between the technologies.

After in silico simulations and in vitro successes have occurred, are we open to a beneficial "superior" genomebeing introduced in to the population? Currently the ability to genetically screen both parents, and selectively remove certain genes responsible for characteristic traits (e.g. hair and eye colour, and even control the sex of the offspring) during *in vitro* fertilisation (IVF) thus creating 'model children' is already in practice[80-82]. There are vast ethical considerations behind IVF ranging from conquering infertility to the health risks on the mother or child but like any technology, IVF is now long past its introductory phase and only being further studied, developed and improved [83-89].

Can our knowledge of species wide genomes coupled with in silico and in vitro technologies provide a strong enough platform for drug development void of animal testing?

3. HuMANe TRIALS FOR huMANe PROGRESS

The idea of subjecting harmless animals to chronic conditions in order to forcefully study disease processes not for veterinary science but our own desperation, is a cruel practice. Forcing cigarette smoke for pulmonary lung cancer and emphysema studies, to inflammatory intestinal diseases, acute and chronic kidney diseaseto genetic manipulations for rapid tumour development, to cardiovascular and neurodegenerative disease introduction in animals for our benefit is a shameful practice with a lot of preclinical research in these animals ending in failure and irrelevant progression [90-93]. In clinical testing laboratories, animals are isolated from their groups and used as tools regardless of their natural instincts [94-96].Animal models are used for different purposes in drug development particularly to obtain initial safety data or proof of concept that a targeted cell or molecule has a relevant role in the pathogenic process [97]. However, there is increasing evidence that preclinical research in animal models fail to reproduce promising effects when tested in human patients [97-99].The lack of genetic variation and difference in environmental factors with inbred laboratory mice contrasts with the genetic and environmental complexity of human patients [97, 100]. The pathogenic mechanisms operating in the animal model and the human disease may differ.Biological treatment agents have high species specificity and lack significant cross-reactivity across species. The tested therapeutic agent can have opposite effects in patients compared to the animal model, for example penicillin lacks a toxicological response in mice, but has severe toxicity in guinea pigs or hamsters, and so depending on the animal model used, the outcome could have significant differences [101]. The timing of the treatment can differ between preclinical and clinical tests [97, 102-111].

It is clear that no animal is an exact mimic of a human being but us, and so no other animal but us should undergo treatments for our own benefit. Granted medical progress has been propelled forwards through animal research and anybody who has

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benefited from successful animal research has grounds to differ; but are we ready to value all life with as much consideration as we have for our own. Findings from human patients give a much clearer, relevant and specific picture of developmental and disease pathways [64, 92, 112-115]. There are many who undergo drug trials either voluntarily or due to no other choice having reached a "terminal" state. Could these same individuals undergo genetic modifications using knock-in/knock-out genome editing technologies towards understanding and eradicating disease states, currently not possible through existing medical or pharmaceutical means?

Can genetic technologies within ethical and social boundaries, be used towards genetic manipulation of the human genome potentially eradicating disease states currently only slowed down through medical inhibition, thus evolving and creating a more stable organism?

4. CONCLUSION

Given the vast knowledge and advances made in scientific and human progress over the last century, one thing is evident, our desire for a future has considered not much else but ourselves. How many other life forms need to be sacrificed in order to maintain the survival of one human life (every day)? Desperation to survive coupled with idealistic values and centuries worth of countless wars, have painted us as a species with little self-control over our actions resulting in devastating consequences towards all life. Our current success of survival, with a population exceeding 7 billion, is putting immense pressure leaning towards an extinction crisis. It is safe to say we should slow down on old ways and move forward much more consciously aware of the network connecting all life on this planet. Is it possible for us to see infinite potentials yet not pursue all of them depending on the cost of those goals? Can we refrain from eating "forbidden fruits?"

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